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Apomorphine-induced disruption of prepulse inhibition that can be normalised by systemic haloperidol is insensitive to clozapine pretreatment

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Abstract *Rationale:* Prepulse inhibition (PPI) of startle refers to the phenomenon in which a weak prepulse attenuates the startle response to a succeeding intense stimulus. PPI can be disrupted by systemic apomorphine in animals, and reduced PPI has been consistently reported in schizophrenia patients. The ability of the atypical antipsychotic clozapine to reverse apomorphine-induced PPI deficit has been demonstrated in the rat, but has not yet been tested in the mouse. The present study was designed to fill this gap. *Objective and results:* We investigated the efficacy of clozapine in reversing apomorphine-induced (2.0 or 2.5 mg/kg, SC) PPI deficit in C57BL6 mice. Clozapine failed to restore PPI disruption in apomorphine-treated mice in two independent laboratories across two dose ranges (1–3 mg/kg, IP, or 3–30 mg/kg, PO), whereas the typical antipsychotic haloperidol (1 mg/kg, IP) completely normalised PPI performance. *Conclusions:* Unlike the rat, apomorphine-induced PPI disruption in mice might be instrumental in distinguishing between typical and atypical antipsychotic drugs. This also lends further support to the suggestion that the neuropharmacology of PPI is not identical in the two rodent species.

Keywords Apomorphine · Clozapine · Haloperidol · Mice · Prepulse inhibition · Schizophrenia

Introduction

Prepulse inhibition (PPI) refers to the reduction in startle reaction towards a startle-eliciting “pulse” stimulus when it is shortly preceded by a sub-threshold “prepulse” stimulus (Hoffman and Searle 1965). Dopamine agonist-induced PPI disruption is often employed as an animal model of sensorimotor gating deficits in schizophrenia, as PPI is similarly impaired in schizophrenia patients (Braff et al. 2001). More importantly, such drug-induced deficits can be ameliorated by antipsychotic drugs in the rat (Braff et al. 2001).

The pharmacology of PPI has been extensively studied in the rat (see review by Geyer et al. 2001). Nevertheless, parallel investigations in mice have only begun recently with the availability of genetically modified mice, and with it the opportunity to study the genetic basis of sensorimotor gating. However, the possibility that the molecular neuropharmacology of PPI might differ between the rat and the mouse has been raised (Geyer et al. 2002; Ralph-Williams et al. 2002, 2003). Confirmation of the predictive validity of an animal model requires the model to be sensitive to known clinically effective drugs. This has been firmly established in the rat with both typical and atypical neuroleptic drugs, but equivalent confirmation is still lacking in the mouse. Hence, a clear consensus has yet to be established in the mouse, in particular, with respect to atypical neuroleptic drugs.

One strategy to evaluate potential antipsychotic property is to assess a compound’s ability to enhance PPI in the absence of any treatment-induced deficit (Depoortere et al. 1997; Ouagazzal et al. 2001). A number of typical and atypical neuroleptic compounds (including haloperidol, risperidone, and clozapine) have been shown to enhance PPI in different mouse strains when administered on their own (Curzon and Decker 1998; Olivier et al. 2001; Ouagazzal et al. 2001; Geyer et al. 2002). However, unlike the typical antipsychotic drug haloperidol (Curzon and Decker 1998; Furuya et al. 1999), the efficacy of atypical antipsychotic drugs to reverse apomorphine-induced PPI deficit has not been convincingly demon-

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strated in the mouse. The effectiveness of clozapine in reversing apomorphine-induced PPI disruption has been well established in the rat; however, equivalent demonstration in the mouse is still lacking.

The present study was designed to address this in C57BL6/J mice. C57BL6/J mice exhibit robust PPI, which can be disrupted by systemic apomorphine (Curzon and Decker 1998; Varty et al. 2001). This mouse strain is also widely used in murine behavioural testing, and is increasingly popular amongst behavioral geneticists given its superior performance across a wide spectrum of behavioral assays in comparison to other strains, e.g. those from the 129 family.

Two independent experiments were conducted in two laboratories (Zurich and Basel). Each experiment examined three doses of clozapine given prior to apomorphine administration in comparison to apomorphine or vehicle treatment alone. Experiment 1 (in Zurich) also included two doses of haloperidol: with one dose serving as positive control (at 1 mg/kg, IP), and a lower dose as a negative control (at 0.2 mg/kg, IP). These doses were selected based on pilot studies performed in the Zurich laboratory.

Materials and methods

Subjects. The subjects were male naive C57BL6/J mice, weighing 28–35 g. For experiment 1, they were obtained from the in-house specific-pathogen-free (SPF) breeding facility in the Zurich laboratory. For experiment 2, they were obtained from Iffa Credo (France). The animals were caged in groups of three or four littermates under a reversed light–dark cycle (lights on 2000–0800 hours) in a temperature- ($21 \pm 1^\circ\text{C}$) and humidity- ($55 \pm 5\%$) controlled animal facility, with ad lib food and water. In the allocation of subjects to treatment groups, mice derived from the same litters were always allocated into different groups, so as to minimize possible confounds due to litter effects (Zorrilla 1997). Experiment 1 was carried out during the dark phase of the light–dark cycle, and experiment 2 during the light phase. All procedures involved were in agreement with Swiss regulations for animal experimentation.

Apparatus. These consisted of a set of two (Zurich), or eight (Basel) acoustic startle chambers (SR-LAB; San Diego Instruments, San Diego, Calif., USA), each comprising a non-restrictive cylindrical enclosure made of clear Plexiglas attached horizontally on a mobile platform, which was in turn resting on a solid base inside a sound-attenuated isolated cubicle. A high-frequency loudspeaker mounted directly above the animal enclosure inside each cubicle produced both a continuous background noise of 65 dB_A and various acoustic stimuli in the form of white noise. Vibrations of the Plexiglas enclosure caused by the whole-body startle response of the animal were transduced into analogue signals by a piezoelectric unit attached to the platform. These signals were then digitized and stored by a computer. A total of 130 readings were taken at 0.5-ms intervals (i.e. spanning across 65 ms), starting at the onset of the startle stimulus in pulse-alone and prepulse-plus-pulse trials, and at the onset of the prepulse stimulus in prepulse-alone trials. The average amplitude over the 65 ms was used to determine the stimulus reactivity. The sensitivity of the stabilimeter was routinely calibrated to ensure consistency between chambers and across sessions.

Procedures. The two experiments were performed in two independent laboratories (experiment 1: the Swiss Federal Institute of

Technology Zurich, and experiment 2: F. Hoffmann-La Roche Basel) in order to have two independent sets of data. In the demonstration of PPI of the acoustic startle reflex, subjects were presented with a series of discrete trials comprising a mixture of four types of trials. These included pulse-alone trials, prepulse-plus-pulse trials, prepulse-alone trials, and trials in which no discrete stimulus, other than the constant background noise, was presented. A reduction of startle magnitude in prepulse-plus-pulse trials relative to that in pulse-alone trials constitutes PPI. The pulse stimulus employed was 120 dB_A in intensity and 40 ms in duration.

Prepulses of various intensities were employed: 69, 73, 77, 81, and 85 dB_A in experiment 1 or 69, 73, 77, and 81 dB_A in experiment 2, which corresponded to 4, 8, 12, 16, and additionally 20 dB_A (experiment 1 only) above background. The duration of prepulse stimuli was 20 ms. The SOA (stimulus onset asynchrony) of the prepulse and pulse stimuli on prepulse-plus-pulse trials was 100 ms.

A session began with the animals being placed into the Plexiglas enclosure. They were acclimatised to the apparatus for 2 min before the first trial began. The first six trials consisted of startle-alone trials only, and they served to habituate and stabilise the animals' startle response. Subsequently, there were 120 trials comprising 12 blocks of trials each. Each block consisted of one trial of each of the following trial types: startle-alone, prepulse-plus-pulse trials of each of the 4–5 levels of prepulse, prepulse-alone of each of the 4–5 levels of prepulse, and no stimulus (i.e. background alone). The interval between successive trials was variable, with a mean of 15 s (ranged 10–20 s). The test session lasted for approximately 40 min in experiment 1, and 30 min in experiment 2.

Drugs. All solutions were freshly prepared on the day of testing, and were administered in a volume of 5 ml/kg. Apomorphine HCl (APO, obtained from Sigma Chemicals, St Louis, USA) was dissolved in sterile 1% ascorbic acid (VitC, pH 3.2) to achieve the desired concentration (2.0 and 2.5 mg/kg for experiments 1 and 2, respectively; both at pH 3.1). Haloperidol (HAL, obtained from Janssen-Cilag, Baar, Switzerland) was prepared from 5-mg ampoules, in which the drug was present in 1 ml of solvent containing 6 mg lactic acid. This was diluted with sterile 0.9% NaCl solution to obtain the required concentration of either 0.2 or 1.0 mg/kg (final pH of 5.5). Clozapine (CLZ, obtained from F. Hoffmann-La Roche, Switzerland) was prepared differently for the two experiments, depending on the chosen routes of administration. In experiment 1, it was first dissolved in 0.1 N HCl in 0.9% saline solution and then neutralised to pH 5.5 with Na₂CO₃ to obtain the three required doses: 1.0, 2.0, and 3.0 mg/kg. In experiment 2, clozapine was micro-suspended in a mixture of Tween-80 (0.3%)–saline (0.9%) to obtain the three required doses: 3.0, 15.0, and 30.0 mg/kg.

The first injection (HAL, CLZ, or the corresponding vehicle solution) was made 60 min before testing, either via the intraperitoneal route in experiment 1 or per os (PO) in experiment 2. In experiment 1, half of the animals receiving vehicle treatment as their first injection were given 0.9% saline/lactic acid, pH 5.5 (vehicle for haloperidol), and the other half 0.1 N HCl/0.9% saline, pH 5.5 (vehicle for clozapine). In experiment 2, the animals receiving vehicle as their first treatment were all given the Tween-80 (0.3%)–saline (0.9%) mixture.

The second injection was either APO or VitC, and was administered 15 min before testing via the subcutaneous (SC) route in both experiments.

Data analysis. The test trials following the first six acclimatisation trials of startle-alone trials were sub-divided into different trial types and the average values obtained for each individual animal. Percentage prepulse inhibition (%PPI) at each level of prepulse intensity was then calculated with the formula: [(pulse-alone – prepulse-plus-pulse)/pulse-alone \times 100%].

Startle reactivity across the 12 pulse-alone trials (excluding the first six startle-alone trials), and percent PPI were separately analysed using two-way ANOVAs with the between-subjects treatment factor, and the appropriate repeated measures (prepulse intensities or blocks of two trials). Significant effects were further

examined by post hoc pair-wise comparisons using the Student-Newman-Keuls procedure.

The number of subjects in each of the seven groups in experiment 1 were VEH/VitC, $n=9$; VEH/APO, $n=9$; CLZ1/APO, $n=10$; CLZ2/APO, $n=9$; CLZ3/APO, $n=10$; HAL0.2/APO, $n=10$; HAL1.0/APO, $n=10$. The number of subjects in each of the five groups in experiment 2 were VEH/VitC, $n=9$; VEH/APO, $n=9$; CLZ3/APO, $n=9$; CLZ15/APO, $n=6$; CLZ30/APO, $n=6$.

Results

Experiment 1 (Zurich)

The presence of a prepulse reduced the startle response to the succeeding startle stimulus, and constituted the PPI effect. Increasing prepulse intensity resulted in stronger PPI, and this tendency was evident in all groups. However, systemic apomorphine (2.0 mg/kg) attenuated the expression of PPI, and this effect was fully reversed by the pretreatment of haloperidol at the dose of 1.0 mg/kg. This contrasts with the lack of any effect by clozapine pretreatment across all three doses examined, and the lower dose of haloperidol (0.2 mg/kg), which was not expected to alleviate the disruption of PPI induced by apomorphine (see Fig. 1a).

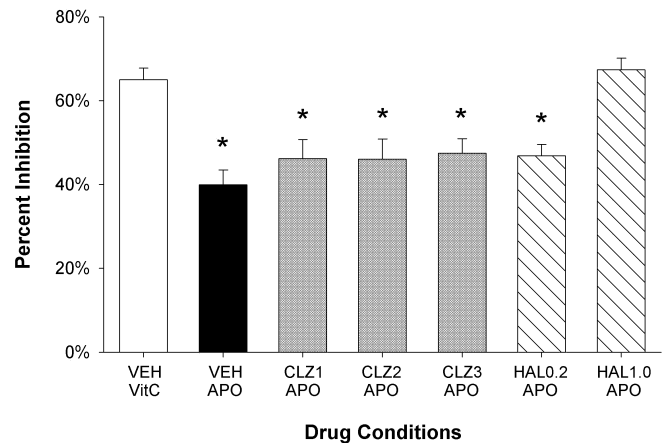
These results were confirmed by the ANOVA of %PPI, which yielded a significant main effect of treatment [$F(6,60)=4.38$, $P<0.005$], of prepulse levels [$F(4,240)=180.02$, $P<0.0001$], and their interaction [$F(24,240)=2.05$, $P<0.005$]. Pairwise comparisons indicated that PPI was significantly reduced in VEH/APO, CLZ1.0/APO, CLZ2.0/APO, CLZ3.0/APO, and HAL0.2/APO groups relative to the VEH/VitC control group [all P -values <0.05] and the positive control group HAL1.0/APO. The VEH/VitC and HAL1.0/APO groups did not differ from each other.

Separate analysis of startle reactivity scores obtained in pulse-alone trials revealed no significant main effects of treatment or two-trial blocks. The mean \pm SEM startle reactivity (in arbitrary units) for each of the groups is summarised in Table 1.

Experiment 2 (Basel)

Experiment 2 yielded a similar pattern of results as experiment 1 in terms of the disruption of PPI by apomorphine and the lack of an effect by clozapine in antagonising this effect. The levels of PPI in the VEH-VitC and in the VEH-APO groups were highly comparable between the two experiments, despite the differences between the two experiments in terms of reactivity scores. The latter might be attributed to a difference in the reference value employed in the two laboratories in the calibration of the startle detection mechanism. The level of PPI observed in the three groups receiving clozapine pretreatment (3–30 mg/kg) did not differ from the VEH-APO animals. Thus, clozapine in this dose range failed to

(a) Experiment 1 (Zurich)



(b) Experiment 2 (Basel)

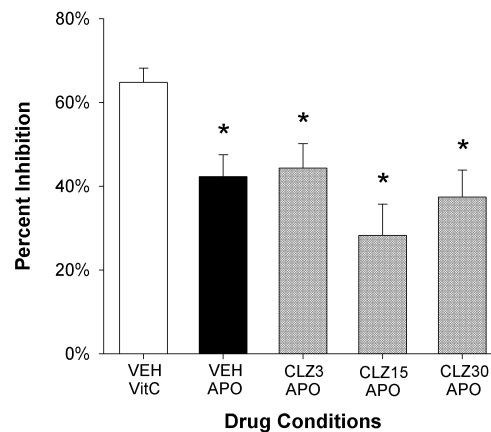


Fig. 1 **a** Experiment 1 conducted in Zurich. Average percent prepulse inhibition (%PPI) across the five different prepulse intensities measured in mice previously treated with 2.0 mg/kg apomorphine (APO), vitamin C (VitC), 1–3 mg/kg clozapine (CLZ), and 0.2–1.0 mg/kg haloperidol (HAL) assigned to one of seven experimental conditions. Error bars \pm SEM. $n=9$ –10 per group. **b** Experiment 2 conducted in Basel. Average percent prepulse inhibition (%PPI) across the four different prepulse intensities measured in mice previously treated with 2.5 mg/kg, SC APO, VitC, 3–30 mg/kg CLZ. Error bars \pm SEM. $n=6$ –9 per group. *Denotes significant difference from VEH-VitC control ($P<0.05$), based on Newman-Keuls post hoc comparisons

antagonise apomorphine-induced PPI disruption (see Fig. 1b).

These conclusions were supported by ANOVA of %PPI, which revealed a significant main effect of treatment [$F(4,34)=5.65$, $P<0.001$] and of prepulse intensities [$F(3,102)=74.33$, $P<0.0001$], but not of their interaction [$F(12,102)=1.165$, $P=0.32$]. Post hoc Newman-Keuls analysis confirmed the impression that PPI was disrupted in all apomorphine-treated groups compared to the VEH-VitC controls (all P -values <0.05). The magnitude of PPI did not differ among the four apomorphine-treated groups, regardless of whether they were pretreated with clozapine.

Table 1 Mean reactivity (\pm SEM) on pulse-alone trials in each experimental condition of experiments 1 and 2

	Sample size	Mean startle reactivity (\pm SEM)
Experiment 1 (Zurich)		
VEH/VitC	9	132.17 \pm 8.11
VEH/APO	9	140.89 \pm 14.00
CLZ (1 mg/kg, IP)/APO	10	113.94 \pm 16.89
CLZ (2 mg/kg, IP)/APO	9	104.81 \pm 13.86
CLZ (3 mg/kg, IP)/APO	10	109.32 \pm 10.60
HAL (0.2 mg/kg, IP)/APO	10	146.53 \pm 14.70
HAL (1.0 mg/kg, IP)/APO	10	163.80 \pm 15.85
Experiment 2 (Basel)		
VEH/VitC	9	104.47 \pm 16.66
VEH/APO	9	66.14 \pm 10.60*
CLZ (3 mg/kg, PO)/APO	9	60.06 \pm 10.96*
CLZ (15 mg/kg, PO)/APO	6	30.81 \pm 4.67*
CLZ (30 mg/kg, PO)/APO	6	22.53 \pm 3.13*

* Denotes significant difference from VEH–VitC control ($P<0.05$), based on Newman–Keuls post hoc comparisons

Analysis of startle reactivity on startle-alone trials revealed a significant main effect of treatment [$F(4.34)=7.13$, $P<0.001$]. The mean \pm SEM startle reactivity for each of the groups is summarised in Table 1. Post hoc Newman–Keuls comparisons indicated that reactivity on startle-alone trials was significantly reduced in all groups in comparison to the VEH/VitC group (all P -values <0.05).

Discussion

The present study provides a direct test of whether apomorphine-induced PPI disruption can be antagonised by the atypical antipsychotic clozapine, in C57BL/6J male mice. The clear absence of an effect here was demonstrated in two independent laboratories using two different routes of administration, and being conducted in different phases of the light–dark cycle. Between the two experiments, clozapine was examined across a dose range from 1.0 to 30.0 mg/kg. This contrasted with the efficacy of the typical antipsychotic haloperidol, which completely restored PPI performance to a normal level at the appropriate dose (i.e. dose-dependent). These findings are of significance to behavioural phenotyping in genetically modified mice, especially in the application of the PPI paradigm as assessment of schizophrenia-related mutations.

In both experiments, the null effect of clozapine was clear and was not associated with any tendency of a dose-dependent effect. Following experiment 1, we attempted the use of higher doses of clozapine in the Zurich laboratory using the IP route (6–10 mg/kg), but these were accompanied by excessive sedation and a massive reduction in startle reactivity as described earlier by others (Ouagazzal et al. 2001; Olivier et al. 2001). In experiment 2, the higher doses administered via the PO route still led to a significant reduction of startle reactivity. Hence, clozapine was behaviorally effective in the present study,

yet it remained totally ineffective in counteracting the disruptive effect of apomorphine on PPI.

The present study examined only one mouse strain, and the generalization of the present findings to other mouse strains needs to be further explored. The possibility that the present null effect of clozapine is unique to the C57BL/6J strain cannot be excluded, given growing evidence that the expression of PPI and its sensitivity to drug manipulation can vary significantly across different mouse strains (Varty et al. 2001; Geyer et al. 2002). Even in the rat, the effectiveness of clozapine in reversing apomorphine-induced PPI disruption is at least under some circumstances strain-dependent, although the ability of haloperidol to antagonise apomorphine-induced PPI disruption is largely independent of rat strains (see Geyer et al. 2001 for review). Considering the common use of the C57BL/6J mouse strain, the present findings should be noted by researchers in the field of murine behavioral phenotyping.

Nonetheless, the present null findings need to be considered against (i) the positive effect of clozapine in the rat, and (ii) the efficacy of clozapine in enhancing PPI when administered alone in the mouse (e.g. Ouagazzal et al. 2001).

With respect to the first, there has already been a recent indication that the pharmacology of PPI might differ between rats and mice as revealed by the difference in the dopaminergic modulation of PPI (Ralph-Williams et al. 2003). These authors suggested that dopamine D₁-family receptors may play a more prominent role in the modulation of PPI in mice, while the D₂-family receptors appear to be more critically involved in rats. Whether this particular difference alone could satisfactorily account for the present ineffectiveness of clozapine remains to be further evaluated.

As for the second, two recent reports are of particular relevance. First, Dirks et al. (2003) have shown that clozapine was not only ineffective in enhancing PPI when administered alone, but it can even disrupt PPI. This casts some doubt as to whether clozapine-induced PPI enhancement is a robust finding in the mouse. Second, it is noteworthy that Ralph-Williams et al. (2003) have reported that the selective D₁ antagonist, SCH23390, can reverse apomorphine-induced disruption of PPI, and yet does not enhance PPI when administered alone. Thus, at least in mice, PPI enhancement and reversal of apomorphine-induced PPI disruption do not always go hand in hand. With the use of both experimental preparations, PPI in mice might be useful in dissociating the underlying neuro-pharmacological mechanisms between the two phenomena, and potentially in capturing the typical–atypical dichotomy of antipsychotic drugs.

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